Insecticidal and Repellent Properties of Nine Volatile Constituents of Essential Oils against the American Cockroach, *Periplaneta americana* (L.)

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Abstract: The toxic and repellent properties of nine major constituents of essential oils, comprising benzene derivatives and terpenes, were evaluated against *Periplaneta americana* (L.). Contact and fumigant toxicities to adult females and repellency to nymphs were determined. The decreasing order of knockdown activity via contact was methyl-eugenol > isosafrole = eugenol > safrole. The killing effect via contact was in the order eugenol = methyl-eugenol = isosafrole > safrole. Fumigant toxicity was only observed for safrole and isosafrole, with safrole being the more potent. Isoeugenol and the tested terpenes had neither contact nor fumigant toxic effect. The decreasing order of repellency to nymphs was safrole > isosafrole > methyl-eugenol = α-pinene > eugenol > isoeugenol. The benzene derivatives were generally more toxic and repellent to *P. americana* than the terpenes. The distance of the side chain double bond from the aromatic ring and the substitution of a methoxy group to these compounds appeared to be important determinants of their toxicity and repellency. © 1998 Society of Chemical Industry

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Key words: benzene derivatives; terpenes; cockroach; contact toxicity; fumigant toxicity; repellency

1 INTRODUCTION

Synthetic insecticides are currently used for the control of pest cockroaches all around the world. However, their widespread use often suffers from the disadvantages of residual toxicity, health hazard to humans, and development of resistance in several pest species.[1–3] As reviewed by Cornwell,[2] numerous reports have been published on cockroach resistance to various commercial insecticides. Recent concerns with human health and environmental safety, as well as prevalence of insect resistance to existing insecticides have prompted a revival of interest in plant-derived insecticides.[4] Among many secondary metabolites of plants, essential oils and their constituents have received considerable attention in the search for new pesticides,[5] and have been found to possess insecticidal activities.[6,7] For example, a hexane extract of clove (*Syzygium aromaticum* (L.) Merr. and Perry) is toxic to the stored-product beetles, *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch.[8] Essential oil of clove is also toxic to *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (F.),[9] while garlic

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oil[10] and nutmeg oil[11] are insecticidal to *T. castaneum* and *S. zeamais*. Oil extracted from the edible fruit of *Dennettia tripetala* (L.) is toxic to the adults and nymphs of the American cockroach, *Periplaneta americana* (L.).[7]

The volatile components of essential oils can be classified into four main groups: terpenes, benzene derivatives, hydrocarbons and other miscellaneous compounds.[12] They can easily be purified from plants such as nutmeg, clove, star anise and citrus.[13] Owing to their odorous properties, these compounds are widely used in the fragrance and flavour industries for producing food flavourings, cosmetics and detergents.[14] The nine constituents selected for the present study (Fig. 1) are benzene derivatives (safrole, isosafrole, eugenol, methyl-eugenol and isoeugenol) and terpenes (cineole, *p*-cymene, limonene and α-pinene). Some of these compounds exhibit various bioactivities against insects. For example, limonene inhibits the development and growth of offspring from oothecae of the German cockroach, *Blattella germanica* (L.).[15] Antifeedant and growth inhibitory activities to the adults and larvae of *T. castaneum* have been observed for α-pinene,[16] safrole and

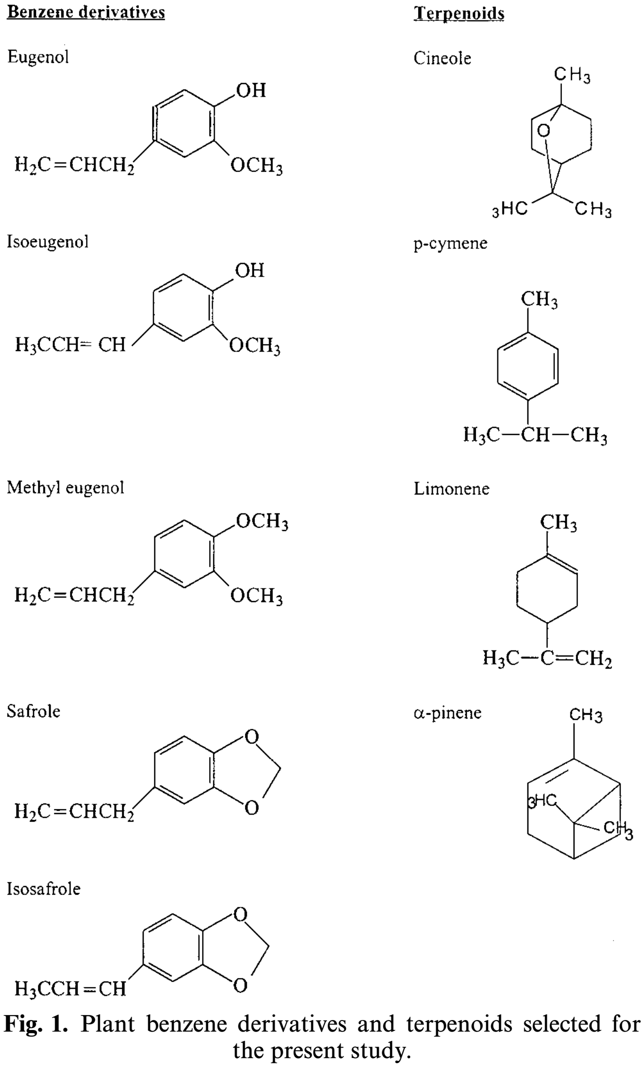

Fig. 1. Plant benzene derivatives and terpenoids selected for the present study.

isosafrole (Huang, Y., Ho, S. H. and Kini, R. M., unpublished). Furthermore, both safrole and isosafrole exhibit toxic and antifeedant effects on *S. zeamais*. Despite the various reports on the insecticidal properties of these compounds against several insects, their bioactivities against the American cockroach, *P. americana*, has not been determined. The present study was therefore initiated to investigate the contact and fumigant toxicity as well as repellency of these chemicals to *P. americana*, the prominent species of cockroach in homes, rubbish dumps and sewage systems.[17]

2 EXPERIMENTAL

2.1 Insects

Adult female American cockroaches were used in both contact toxicity and fumigant toxicity tests. They were reared in cages in the insectary at 25(±2)°C, 64(±5)% RH on water and mouse food pellets. For the repellency test, first-instar nymphs (one to three days old) hatched from oothecae at 30(±1)°C, 75(±3)% RH in incubators were used. These nymphs were removed from the master colony daily and kept in the insectary at 25(±2)°C, 64(±5)% RH on water and mouse pellets.

2.2 Chemicals

Eugenol, safrole, α-pinene, limonene and cineole were obtained from Sigma Co. (UK). Isoeugenol, methyl-eugenol, isosafrole and *p*-cymene were obtained from Aldrich Co. (UK). These concentrates (>97% purity) were serially diluted with analytical grade acetone to appropriate concentrations.

2.3 Bioassays

2.3.1 Contact toxicity

Groups of 10 insects were confined using Fluon GPI lined glass filter funnels (ring diameter 15 cm) inverted over glass plates. Filter papers (Whatman No. 1, 15 cm) were each impregnated with 2·5 ml of the appropriate chemical solution. They were then air-dried for 30 min before being placed between the inverted glass filter funnels and glass plates prepared earlier. This allowed the insects to come into contact with the treated papers. At least six concentrations were tested per chemical and this was replicated at least four times. Acetone was used as control. The set-up was kept at 30(±1)°C and 75(±3)% RH in incubators for 24 h, following which knockdown was recorded. The criterion for knockdown was the inability of insects lying on their backs to right themselves when touched on the

abdomen with a pair of forceps. Each batch of insects was then transferred to clean glass jars with mouse pellets and water. Mortality was recorded daily until end-point mortality was reached after 96 h.

2.3.2 Fumigant toxicity

Prior to the experiment, 10 insects per replicate were placed in screw-topped glass jars (23 cm height × 17 cm diameter), lined with a thin film of Fluon to prevent insects from climbing up the glass wall. Filter papers (11 cm, Whatman No. 1) were each impregnated with 1·3 ml of an appropriate concentration of the test chemical solution, and acetone was used as control. The treated filter papers were then air-dried for 5 min. They were placed over the mouths of the glass jars and sealed tightly with caps. The set-up was then kept at 30(±1)°C and 75(±3)% RH in incubators for 24 h after which the number of cockroaches knocked down was determined. The treated filter papers were removed along with the caps and replaced with fine-mesh gauze to confine the insects within the glass jars containing mouse pellets and water. At least six concentrations were tested per chemical and this was replicated at least five times. The criterion for knockdown for this experiment was the same as that used in the contact toxicity study.

2.3.3 Repellency test

Repellency was tested according to a linear track olfactometer assay based on that of Sakuma and Fukamm[18] with some modifications. The apparatus consisted of an odour source, two traps, pipe-work made of transparent Plexiglass, a pot with test insects and wirework. All experiments were carried out in fume cupboards at room condition of 25(±2)°C and 64(±5)% RH. Briefly, 1 ml of chemical solution in acetone was applied to each filter paper (5 × 40 cm, Whatman No. 1) as uniformly as possible with a glass pipette. Filter papers were then air-dried for 5 min to evaporate the solvent completely. One hundred American cockroach nymphs were transferred to the pot and allowed to stand for a few minutes, while filter papers were placed in appropriate chambers (one side for control and the other for treatment). Air was drawn in with a vacuum pump to condition the olfactometer. The pot containing the insects was then plugged into the olfactometer with a water bath (45°C) placed underneath it. This modification initiated the insects' climb up the wire during the test. Each experiment was completed within 10 min and insects present in each trap were counted. There were four or five replicates for each concentration and control. Repellency is defined as the percentage of insects that avoided the treated chamber and was calculated[19] as follows:

$$\% \text{ Repellency} = 100 - (100 \times Ns)/(Ns + Nc)$$

Ns = Number of insects trapped in the treatment side
Nc = Number of insects trapped in the control side

2.4 Analyses of Data

For all tests, the concentrations required for 50% and 95% knockdown (KD_{50} and KD_{95} respectively) and end-point mortality reached after 96 h (LC_{50} and LC_{95} respectively) as well as for 50% repellency (RP_{50}) were calculated using probit analysis[20] with Maximum Likelihood Programme software. Values were considered to be significantly different if the 95% fiducial limits (FL) did not overlap. In addition to the RP_{50}, chi-square analysis of each set of data was also performed for the repellency test to examine whether the observed chambers were compatible with the even distribution.[18]

3 RESULTS AND DISCUSSION

The present study was initiated to investigate the potential application of several major constituents of essential oils from plants which have long been used in the pharmaceutical and food industries as cockroach control agents. The toxic and repellent properties of nine volatile constituents, selected based on the functional groups of benzene derivatives (eugenol, isoeugenol, methyl-eugenol, safrole and isosafrole) and terpenes (*p*-cymene, limonene, cineole and α-pinene) against *P. americana* were therefore determined.

3.1 Toxicities

The contact toxicity test using filter paper impregnation revealed that only four compounds, eugenol, methyl-eugenol, safrole and isosafrole, demonstrated contact toxic effects on *P. americana* females (Table 1). The KD_{50} value (0·206 mg cm^{-2}) of eugenol was significantly higher than its LC_{50} (0·148 mg cm^{-2}), although the KD_{95} (0·493 mg cm^{-2}) and LC_{95} (0·422 mg cm^{-2}) values of eugenol were not significantly different, indicating that eugenol had a better killing than knockdown effect at lower concentrations. However, its KD_{95} and LC_{95} values suggest that the knockdown effect of eugenol is as effective as its killing action at higher concentrations. In contrast to eugenol, the LC_{50} (0·187 mg cm^{-2}) and LC_{95} (0·438 mg cm^{-2}) values of methyl-eugenol were significantly greater than its KD_{50} (0·140 mg cm^{-2}) and KD_{95} (0·227 mg cm^{-2}) values respectively, indicating that methyl-eugenol possesses good knockdown activity, although its lethal effect is not strong. It is noteworthy that the KD_{95} of methyl-eugenol is in the same order of magnitude as the KD_{50} of eugenol, indicating that methyl-eugenol is a better knockdown agent than eugenol. As their LC values are not significantly different, both eugenol and methyl-eugenol appeared to have the same killing effect on the insects.

Safrole has a higher LC_{50} (0·335 mg cm^{-2}) than KD_{50} (0·281 mg cm^{-2}), but similar values for LC_{95}

TABLE 1
Contact Toxicity of Nine Constituents of Essential Oils to *Periplaneta americana* Females

(*a*) *Knockdown recorded at 24 h post-treatment*

Chemical	*n*	*KD* (*mg cm*$^{-2}$) (*95% fiducial limits*) *KD*$_{50}$	*KD*$_{95}$	*Slope* ($\pm$*S.E.*)	χ^2	*d.f.*[a]	*H.F.*[b]
Eugenol	280	0·206 (0·188–0·228)	0·493 (0·393–0·738)	4·430 (0·654)	44·729	22	2·485
Methyl-eugenol	350	0·140 (0·131–0·150)	0·227 (0·200–0·280)	7·843 (1·108)	53·289	28	1·853
Safrole	340	0·281 (0·263–0·302)	0·478 (0·427–0·559)	7·156 (0·706)	61·496	28	1·270
Isosafrole	350	0·199 (0·187–0·213)	0·351 (0·315–0·408)	6·680 (0·619)	35·028	28	0·970
Isoeugenol			>0·762				
Cineole, *p*-Cymene, Limonene, α-Pinene			>6.500				

(*b*) *End-point mortality at 96 h post-treatment*

Chemical	*n*	*LC* (*mg cm*$^{-2}$) (*95% fiducial limits*) *LC*$_{50}$	*LC*$_{95}$	*Slope* ($\pm$*S.E.*)	χ^2	*d.f.*[a]	*H.F.*[b]
Eugenol	280	0·148 (0·125–0·166)	0·422 (0·334–0·671)	3·611 (0·64)	37·95	22	1·340
Methyl-eugenol	350	0·187 (0·164–0·234)	0·438 (0·316–0·860)	4·449 (0·82)	53·492	28	2·045
Safrole	340	0·335 (0·316–0·353)	0·464 (0·427–0·537)	11·629 (1·824)	52·851	28	1·660
Isosafrole	350	0·195 (0·183–0·208)	0·334 (0·302–0·385)	7·027 (0·642)	44·345	28	1·083

[a] d.f.: Degrees of freedom.
[b] H.F.: Heterogeneity factor.

(0·464 mg cm^{-2}) and KD$_{95}$ (0·478 mg cm^{-2}), suggesting that it has a better knockdown than killing effect at lower concentrations. On the other hand, as the KD and LC values for isosafrole do not significantly differ from each other, isosafrole is equipotent in knocking down and killing the insects. When safrole and isosafrole are compared in terms of both knockdown and killing actions, isosafrole is the better contact toxicant to *P. americana* females.

A comparison of the KD values (Table 1(a)) of these contact toxicants showed that their potencies as knockdown agents against *P. americana* females were in the order: methyl-eugenol > eugenol = isosafrole > safrole. However, the LC values of these compounds (at 50% or 95% mortality) were not significantly different from each other, with the exception of LC$_{50}$ for safrole, which was significantly higher than the rest (Table 1(b)). Interestingly, isoeugenol and the terpenes did not show any contact toxicity at the same concentration range used for these four benzene derivatives.

Among nine constituents of the essential oils, safrole and isosafrole exhibit fumigant toxicity to *P. americana* (Table 2). As in the case of contact toxicity, differences between KD and LC values were observed for safrole, but not for isosafrole. Safrole has significantly lower KD values than LC values suggesting that it is a better knockdown than killing agent. When compared with isosafrole, safrole has lower KD and LC values than isosafrole. This observation suggests that safrole is a better knockdown than killing agent while isosafrole is equipotent in both actions, an observation similar to that for the contact toxicity of these compounds. However, while isosafrole is a more potent contact toxicant to *P. americana* females, safrole is a better fumigant to these insects. Interestingly, eugenol and its analogues showed low or negligible fumigant toxicity even at con-

TABLE 2
Fumigant Toxicity of Nine Constituents of Essential Oils to *Periplaneta americana* Females

(*a*) *Knockdown recorded at 24 h post-treatment*

Chemicals	*n*	*KD* ($\mu g\ cm^{-2}$) (*95% fiducial limits*) KD_{50}	KD_{95}	*Slope* ($\pm$*S.E.*)	χ^2	*d.f.*[a]	*H.F.*[b]
Safrole	400	0·159 (0·149–0·169)	0·279 (0·254–0·320)	6·776 (0·768)	75·859	34	2·026
Isosafrole	320	0·262 (0·246–0·282)	0·495 (0·427–0·621)	5·980 (0·710)	47·340	26	0·560
Eugenol			>0·750				
Isoeugenol			>7·400				
Methyl-eugenol			>7·100				
Cineole, *p*-Cymene, Limonene, α-Pinene			>0.700				

(*b*) *End-point mortality at 96 h post-treatment*

Chemical	*n*	*LC* ($mg\ cm^{-2}$) (*95% fiducial limits*) LC_{50}	LC_{95}	*Slope* ($\pm$*S.E.*)	χ^2	*d.f.*[a]	*H.F.*[b]
Safrole	400	0·202 (0·190–0·214)	0·400 (0·352–0·483)	5·525 (0·578)	93·646	34	2·370
Isosafrole	320	0·301 (0·278–0·334)	0·630 (0·514–0·889)	5·120 (0·700)	43·970	26	1·030

[a] d.f.: Degrees of freedom.
[b] H.F.: Heterogeneity factor.

centrations above 0·7 mg cm^{-2}. As in the case of contact toxicity, none of the terpenes tested showed any fumigant toxicity to the insects.

Despite the lack of any toxic action of the terpenes in this study, their toxic properties against other insect species have been reported.[15,16,21] Referring to these publications, we can conclude that the terpenes, while being toxic to various species of beetle, are not toxic to *P. americana*.

Our biological data about the tested compounds indicated that a compound which has strong lethal activity cannot exhibit outstanding knockdown activity and *vice versa*.[22,23] This is particularly true for some of the synthetic pyrethroids like resmethrin (good killing with poor knockdown properties) and allethrin (rapid knockdown but poor killing power). Although the exact mode of action of the four contact toxicants (eugenol, methyl-eugenol, safrole and isosafrole) on *P. americana* is unknown, the contact toxicity of these chemicals suggests that they can somehow penetrate the integument of the insects, to act presumably on the nervous or respiratory system to produce the killing effect. As eugenol and methyl-eugenol do not have observable fumigant toxicity, it is reasonable to conclude that they can only exert their killing effects following either their ingestion during grooming or penetration through the integument of the insects. The fumigant effect, in addition to contact toxicity, of safrole and isosafrole suggests that direct contact is not the only means by which these compounds kill the insects.

The chemical structures of the compounds (Fig. 1) may also determine the killing potencies of the test insects. From our results on eugenol and its analogues, contact toxicity decreases when the double bond is closer to the aromatic ring in isoeugenol compared to eugenol and methyl-eugenol. Furthermore, in this group of compounds, the additional methoxy functional group in methyl-eugenol increases the knockdown capability without significantly affecting its lethality. Similarly, fumigant activity decreases when the side chain double bond is closer to the aromatic ring (isosafrole) and a ring closure (safrole and isosafrole) appears to be also important for fumigant toxicity.

3.2 Repellency

Much work has been carried out on cockroach repellents,[19,24–26] which cause directed movement of pests away from treated surfaces. These repellents are useful

in difficult-to-reach hidden places such as electrical and plumbing systems, which may serve as runways for cockroaches and facilitate their dispersal between apartments. Furthermore, non-toxic and relatively volatile repellents may be applied to surfaces through cleaning solutions which protect merchandise in transport and storage, and sensitive equipment from being disrupted by pest insects. For such applications, repellents must have low mammalian toxicity and relatively low residual activity. The repellency of the nine compounds to *P. americana* nymphs is shown in Table 3. Among these compounds, all the tested benzene derivatives exhibit significantly ($P < 0{\cdot}05$) high repelling activity against cockroaches. Among them, the most effective repellent was safrole which exhibited a 50% repellency at a concentration of 8·42 μg cm^{-2} (RP_{50}). This was followed by isosafrole (which had a RP_{50} approximately two-and-a-half times that of safrole), methyl-eugenol, eugenol and isoeugenol in that order. In contrast to the benzene derivatives, cineole, *p*-cymene and limonene show little or no repellent activity to the insects even at concentrations above 200 μg cm^{-2}. However, α-pinene showed the same magnitude of repellency as methyl-eugenol. These results clearly demonstrated that the *P. americana* nymphs exhibited negative chemotaxis to all the benzene derivatives (eugenol, safrole and their analogues) and one terpene (α-pinene).

Although some studies have been done on the chemotactic activity of different insects to plant-derived compounds,[5,26,28] a complete profile on the activities of these compounds to one insect species had not been documented previously. It is well-known that insecticides can act as repellents at high concentrations and as attractants at low concentrations.[29] Although only the repellent properties of nine constituents of essential oils are reported here, it is very likely that these compounds may elicit such biphasic response in the insects when tested at lower dosages. This is particularly true with methyl-eugenol which elicits this biphasic response in *P. americana* nymphs, depending on the concentrations used (Choo, E. W., Pang, F. Y. and Ho, S. H., unpublished). Such attractancy is not novel as extensive studies have previously been reported in the male oriental fruit fly, *Dacus dorsalis* (Hendel).[30–33] There is a possibility that these chemicals could bring about various biological responses in insects. Eugenol, for example, is a repellent against mosquitoes,[34] but is an attractant kairomone for a number of insect pests including the housefly, *Musca domestica* (L.)[35] and the northern corn rootworm, *Diabrotica barberi* (Smith and Lawrence).[36] Unfortunately, the different experimental methods used in these reports cannot be compared with the results obtained in this study.

As shown in Fig. 1, repellent activity of these compounds increases with the distance of the side-chain double bond from the aromatic ring (eugenol and

TABLE 3
Repellency of Nine Constituents of Essential Oils to *Periplaneta americana* Nymphs using a Linear Track Olfactometer

		RP_{50} (95% FL) (μg cm^{-2})	Slope (±S.E.)	χ^2	d.f.[a]	H.F.[b]
Benzene derivatives						
	Eugenol	77·14 (69·13–85·37)[c]	2·443 (0·156)	154·060	23	5·237
	Isoeugenol	131·64 (109·95–156·61)[c]	1·089 (0·097)	219·041	28	6·180
	Methyl-eugenol	53·00 (41·78–65·30)[c]	2·039 (0·212)	84·621	21	4·386
	Safrole	8·42 (6·02–11·26)[c]	0·796 (0·061)	126·415	23	5·806
	Isosafrole	22·71 (17·57–28·92)[c]	0·835 (0·059)	128·654	28	4·683
Terpenes						
	α-Pinene	58·46 (51·03–65·84)[c]	2·434 (0·164)	125·568	28	3·921
	Limonene	>210				
	Cineole	>690				
	p-Cymene	>215				

[a] d.f.: degrees of freedom.
[b] H.F.: Heterogeneity factor.
[c] These chemicals were significantly repellent ($P < 0{\cdot}005$) by chi-square analysis based on an expected null distribution between treated and control chambers.

safrole), substitution with a methoxy group (methyl-eugenol) or the presence of a closed ring structure (safrole and isosafrole). Generally, the benzene derivatives are more effective repellents than the terpenes to *P. americana* nymphs. From these observations, we can suggest that, with the exception of α-pinene, certain functional groups (e.g. methoxy) are important determinants of repellency within this family of compounds.

In conclusion, our results on the bioactivity of nine constituents against *P. americana* showed that the benzene derivatives (i.e. eugenol, methyl-eugenol, isoeugenol, safrole and isosafrole) are better toxicants and repellents to the insects than the monoterpenes (limonene, cineole and *p*-cymene). Only α-pinene exhibits a considerable repellent effect on the nymphs. These findings are promising as cockroach control using these compounds might be feasible and future work is highly recommended in this respect.

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